MOLECULAR ASPECTS OF CORNIFICATION

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A new delineation of the structure of horn fibers has been made possible by x-ray diffraction analysis (1), by layer stripping methods, by layer digestion, and by electron microscopy (2). These methods of investigation have revealed that natural fibers (wool, silk, flax and cotton) have the character of "molecular yarns" (3, 4), which are distinguished by their properties of flexibility and wriggliness.

Electron microscopy has demonstrated that collagen fibers are banded. If such a fiber is made to swell, the ends split like the broken end of a textile yarn (Fig. 1a). The fiber threads are composed of individual, spherical bodies which are evenly distributed through the entire length of the broken fiber (Fig. 1a). This is the so-called molecular level of a collagen fiber.

Similar phenomena are encountered in the earthworm's cuticle (2), (Fig. 1b). The lowest layer contains ridges which are markedly granular or corpuscular. The external surface is covered, however, only by corpuscles which are discrete and evenly distributed. When isolated, these surface corpuscles show distinct "tails" which resemble certain bacteriophages in size and shape.

In previous investigations (5, 6), some unique features of keratin structures of human skin were demonstrated by means of ordinary light microscopy. Cloudy masses were shown to surround horn structures in which corkscrew- and digit-like processes extended. The structures within isolated horn fibrils exhibited the "dove-tail" mechanism. Also, there were transverse bands in individual fibers. The meaning of these observations is not fully understood. It is the purpose of this paper to clarify these features and to attempt to demonstrate the life cycle and the molecular structure of human keratin fibers.

MATERIAL AND METHODS

Scales of normal skin and scrapings from plantar warts and from lesions of psoriasis were prepared for examination with the electron microscope in the following manner: The material was minced in sterile distilled water, or was broken up in 33 per cent alcohol, and the mixture was then centrifuged at low speed for half an hour. The supernatant was fixed on a glass slide with osmic tetroxide vapors and was shadowed with chromium at Tangent one third angle. A collodion membrane was formed, stripped off, and placed on the screen grid for examination. Magnifications of 5800 and 8100 times were used, with further enlargement being obtained by photographic methods.

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RESULTS

I. The electron microscopy of cloudy masses and of digit-like processes; their relationship to the development of fibers (Fig. 2, A–E)

Cloudy masses which vary in intensity surround numerous horn cells and individual fibers. Some portions of these masses are particularly dense (Fig. 2A, B, arrows). These condensation centers appear as roundish bodies

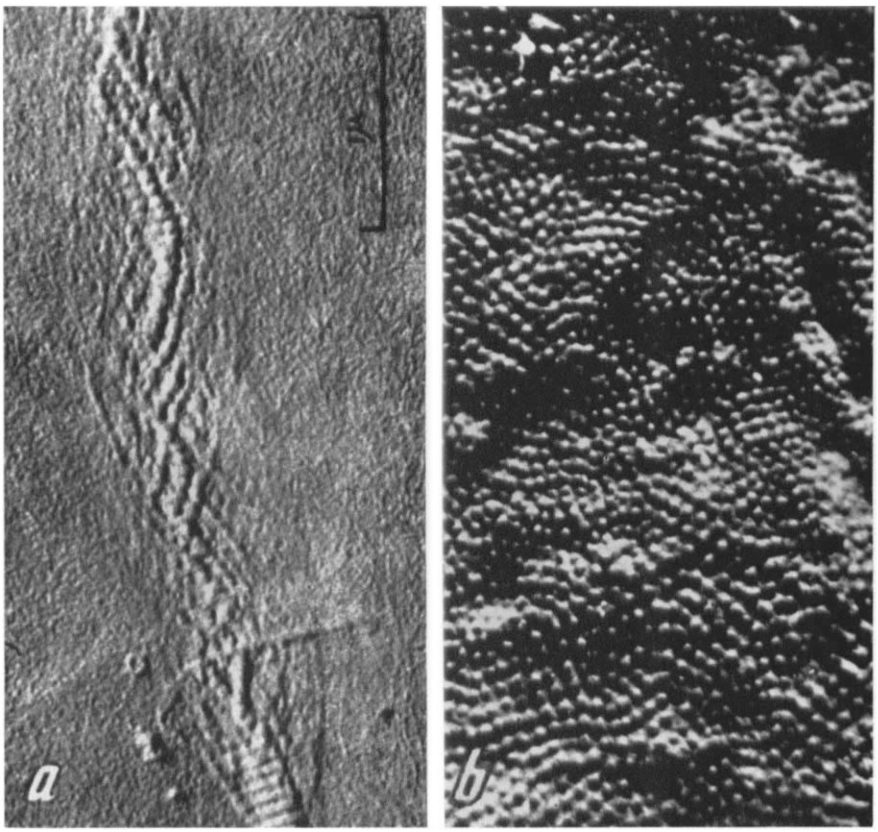

FIG. 1. (a) Electron micrograph of a slightly swollen collagen fibril. Reprinted by courtesy of Dr. R. W. G. Wyckoff (11). (b) Electron micrograph of the external surface of the earthworm's cuticle. Reprinted by courtesy of Messrs. R. Reed and K. M. Rudall (2).

measuring from 24 mμ to 66 mμ by 58 mμ in both diameters (Fig. 2B, arrow) or as longitudinal stripes (Fig. 2C, arrow). There are processes with dichotomous and trichotomous bifurcations (Fig. 2C, arrow). The significance of this phenomenon can be recognized by a study of illustration 2D and 2E. In the upper third of Fig. 2D double arrow a dense area from which a new branch of a fibril appears to originate can be seen. A second branch appears to arise from a condensation point in the lower part of the picture. At its ends, there are two or three digit-like processes (arrow). In Fig. 2E, arrow, the same features can be seen in three areas. The shortest branch (upper arrow) measures 76 mμ, the largest (lower arrows) 312 mμ. The entire fiber *and* the branches are composed of individual corpuscles arranged in uniform rows. The corpuscles measure 7.6 mμ in the fibril and 27 mμ in the processes.

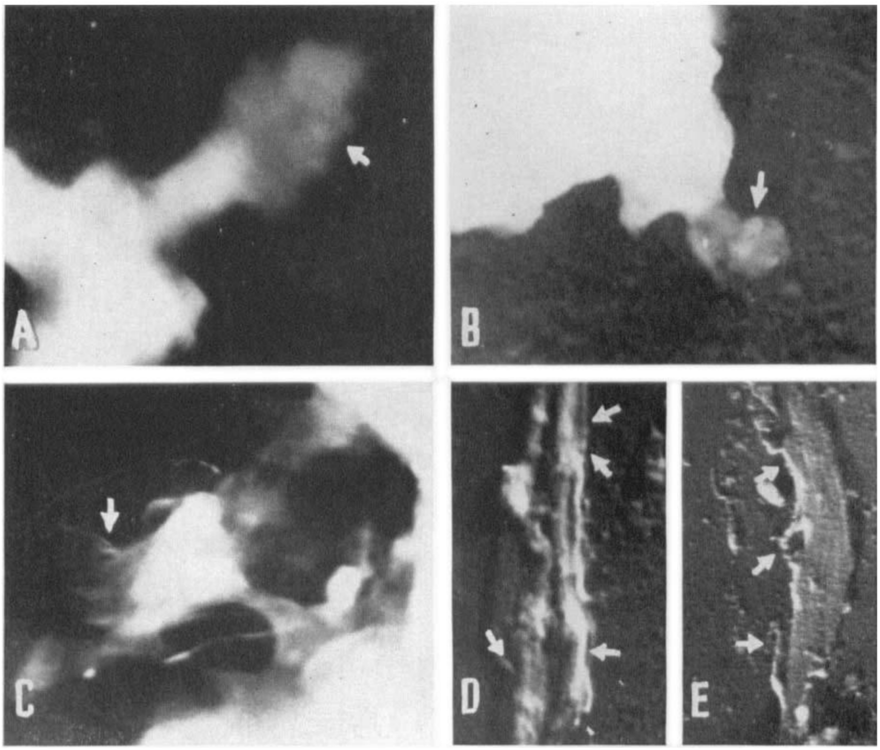

FIG. 2. (A) Plantar wart, magnification ×8100; enlarged to 60000. (B) Plantar wart, ×8100; enlarged ×58000. (C) Plantar wart, ×8100; enlarged ×64500. (D) Normal scales, ×8100; enlarged ×56000. (E) Normal scales, ×8100; enlarged ×62000.

II. Keratin structures exhibiting spiral processes and band-like features (Fig. 2E; Fig. 3A–D: Fig. 4A)

An indication of the spirality of immature horn fibrils is seen in Fig. 2E. It is most marked in the fibrils in Fig. 3A, and Fig. 4A, where they simulate the spiral curve of a watch spring. Other features are seen, such as the early stages of short horn-like and thorn-like processes (Fig. 3A, arrow left). Another marked peculiarity is the occurrence of band-like intervals within a keratin fibril (arrow right). The spirally curved processes of Fig. 3B, C, and D, and the large spiral formations of Fig. 4A, also exhibit the phenomenon of band-like structures (arrows). The individual fragments of the spiral process in Fig. 3C, measure 72 by 115 mμ, those in Fig. 3D, 62 by 103 mμ on the average.

III. Two additional observations (Fig. 4B, C)

Although it was not intended to study differences between normal and pathological cornification, there have appeared several differences between material from plantar warts and normal skin. Fig. 4B, illustrates a keratinized cell from a plantar wart with cornification centers scattered throughout the whole structure. Fig. 4C, is a fragment from a plantar wart which is covered with spherical bodies and with branched filamentous forms (arrows). The triangular structure

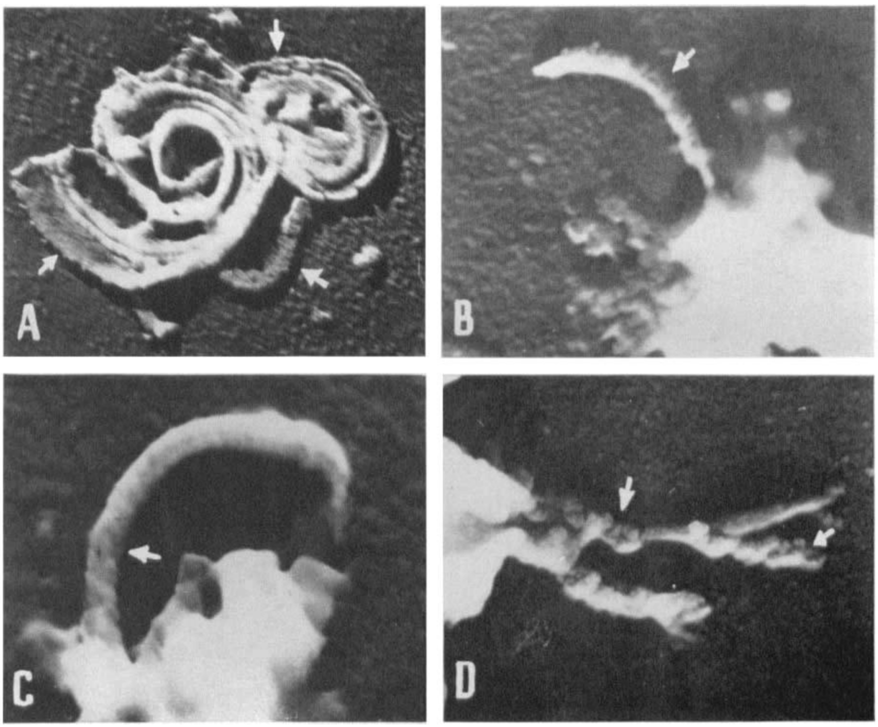

FIG. 3. (A) Psoriasis scales, ×8100; enlarged ×40500. (B) Plantar wart, ×5000; enlarged ×52000. (C) Psoriasis scales, ×8100; enlarged ×48500. (D) Plantar wart, ×5800; enlarged ×46500.

measures 1600 by 1800 by 860 mμ; the smallest corpuscle 28 mμ, the larger branched structure (right arrow) 145 by 450 mμ.

DISCUSSION

Are the multiplicity, variety and almost confusing abundance of unknown structures in collagen fibers and in keratin artefacts due to the mechanical preparation of the specimens and to the electron bombardment? There is general consent that the striation of the collagen fibers is a natural phenomenon. It can be seen in fresh material (7) (Wyckoff, 11, p. 198). The damage produced during the examination of specimens in the electron microscope is not evident in the change of the shape of structures but in the shrinking of the objects which are exposed to dehydration and electron bombardment. For this reason all measurements of electron microscopic photographs are probably not entirely correct (8).

These factors also apply to the critical examination of keratin. The content of water in keratin is estimated at seven to twelve per cent. Keratin is insoluble in water and diluted acids. It is not digestible. These properties of keratin make it resistant to the influence of chemical and physical conditions.

Furthermore, a series of experiments have been carried out on fresh specimens prepared with dissecting needles.

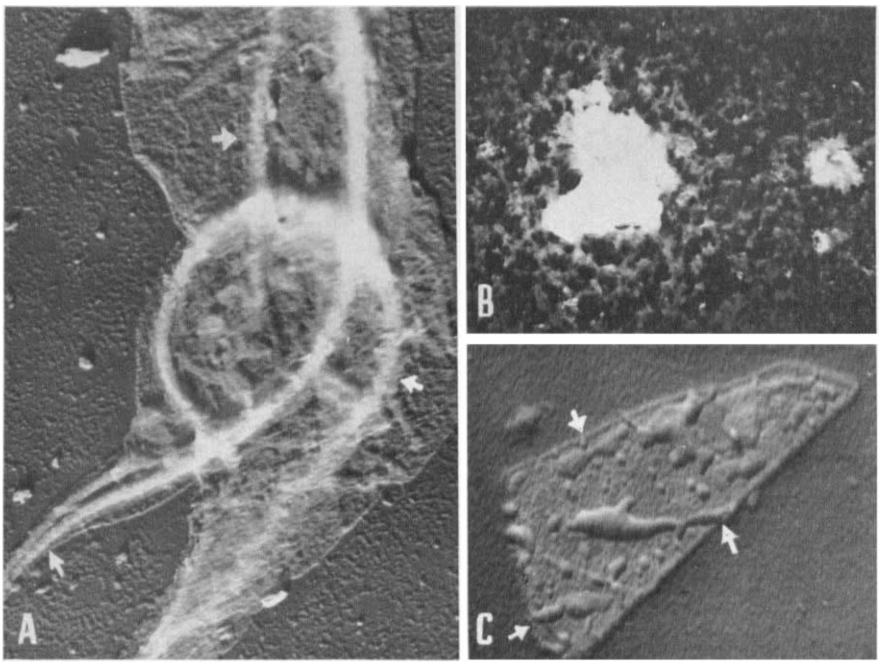

FIG. 4. (A) Psoriasis, ×5800; enlarged to 26000. (B) Plantar wart, ×8100; enlarged to 24300. (C) Plantar wart, ×8100; enlarged to 48600.

The main features of keratin in the electron microscope pictures are: a) cloudy masses around horn substances; b) spiral and; c) straight processes; d) cornified nuclei with patterns of cornification in the protoplasm; e) patterns of cornification in individual horn fibrils.

It is remarkable that all these features are also visible in the ordinary light microscope: a) cloudy masses are reproduced in our publication on cornification (6) (Fig. A, 10, 11, 12); b) spiral processes in cloudy masses (Fig. A, 11, 12); c) straight processes (Fig. A, 11); d) cornified nuclei with evidence of cornification in Figs. B, 6, 8 and D, 2, 3; e) patterns of cornification in individual horn fibrils in Fig. A, 8, 13.

Special attention should be drawn to Fig. B, 8 which shows not only a cornified nucleus but bizarre cornified structures exhibiting branching filaments which resemble in appearance those in Fig. 4C of this publication.

Although these specimens which were fixed in formalin, osmic tetroxide and chromium have not been minced, not broken up in alcohol and not exposed to the electron bombardment of the electron microscope, they show in principle the same structures as in the electron microscope. Therefore it seems reasonable to assume that the electron microscope pictures do not represent artefacts.

It is apparent that every attempt of an interpretation is construed in the light of individual experience and judgment. It remains tentative and subject to modification. This is a modest beginning and it remains necessary to prepare more specimens by the method of layer stripping and digestion.

In the ordinary light microscope the picture is determined by refraction and absorption of light. The electron microscope picture, however, is dependent upon the density of the mass (9). With this interpretation, it can be concluded that the degree of density will be a determinant of the degree of cornification.

It seems reasonable to assume that cloudy masses around keratin substances represent the very first stage of cornification, and that the dense condensation points and the digit-like processes are signs of progression of cornification. Processes may develop either from dense parts as shown in Fig. 2D, or directly from cloudy masses.*

The electron microscope pictures show all stages of spirality, beginning with the spiral form of a very young fibril (Fig. 2E) to the giant spirality represented in Fig. 4A. Whether the bands in keratin fibrils are genuine or whether they represent interspaces resulting from alternating periods in which cornification has occurred is not entirely clear.

During studies on the etiology of psoriasis and allied diseases virus-like, spherical bodies in the scales of eight patients suffering from psoriasis have been demonstrated by electron microscopy (10). Filamentous forms with branches which often are broken up into spherical bodies similar to those pictured by Wyckoff (11) in material presumed to contain the influenza virus have also been photographed. Other viruses may also appear in filamentous forms (12, 13). The lack of inner bodies of the branched filaments in Fig. IV, C, makes it more probable that these figures illustrate progressing keratinization and not virus forms.

Are keratin substances living (14) or dead structures (15)? This problem was vigorously debated half a century ago. The electron microscope pictures of very young and short processes (Fig. 2E) should dispel any doubt as to the very intensive viability of horn substances. They seem to be "the most highly developed cells of the epidermis" (14).

Virchow, the founder of cellular physiology and pathology, described the cell as the last "FORM-ELEMENT" of all living phenomena in health and disease. Electron microscopy has thrown a new light on cellular physiology and pathology. With it, greater internal detail of living substance has created a need for alteration of this concept. The studies on cornification which have been presented in this communication, those by Astbury, Reed and Rudall on the molecular struc-

* An unpublished illustration.

ture of skin and hair, and Wyckoff's observations of the molecular structures in swollen collagen fibers, are based solely on molecular aspects of fibrous proteins, and are phenomena in evidence of a new concept within the realm of normal and pathological histology.

SUMMARY AND CONCLUSIONS

1. New features of cornification have been observed by electron microscopic studies. The molecular aspect of keratin fibrils has been demonstrated. A life cycle of horn fibrils has been suggested.
2. Cloudy masses around keratin substances appear to be the first manifestation of progressing cornification. Digit-like and corkscrew-like processes originate directly in these cloudy masses or derive from condensation points within the cloudy masses.
3. The spirality and wriggliness of certain keratin fibrils has been established by electron microphotography.
4. The presence of bands in young horn fibrils has been demonstrated. They may be genuine bands, or they may be due to the interspace resulting from alternating phases of cornification.
5. Keratin fibrils produce ramifications from which new branches arise. For this reason, keratin is looked upon not as a "dead" but as a growing structure.
6. Keratin fibrils may be composed of discrete and often regularly arranged corpuscles which simulate in their arrangement and size Reed and Rudall's corpuscles on the external surface of the earthworm's cuticle.

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